Research Article

Extraction, Phytochemicals Testing and Antimicrobial Screening of Methanolic Extract of Various Parts of Pomegranate Plant

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ABSTRACT

Punica granatum, commonly known as pomegranate, has been traditionally employed for its therapeutic properties, particularly in the treatment of infectious diseases. This investigation focuses on identification of phytoconstituents from different plant parts and screen for their antibacterial efficacy, thereby providing scientific validation to its ethnomedicinal applications. The peel, seed, leaf, and stem bark of *P. granatum* were collected, shade-dried, and coarsely powdered. Methanolic extract was prepared through maceration process. Qualitative tests were performed to detect the presence of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. Antibacterial activity against *S. aureus* was determined using the agar well diffusion method, with the measurement of zone of inhibition and compared to a standard. Phytochemical screening test results indicated that different plant parts contained varying levels of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. Among extracts, the peel part demonstrated highest antibacterial activity, with a mean inhibition zone of 2.2 cm, followed by leaf (1.8 cm), seed (1.7 cm), and stem bark (1.5 cm). The zone of inhibition for the standard drug (Penicillin G) was found to be 3.2 cm. A noticeable antibacterial activity of the peel extract aligns with its traditional therapeutic usage. The observed differences in the antibacterial potential across the plant parts may be attributed to the varying concentration and nature of bioactive compounds. The significant presence of tannins, flavonoids, and phenolic compounds in the peel could be responsible for its superior antimicrobial effects. These findings confirm the potential of *P. granatum* peel as a natural antibacterial agent and support its ethnomedicinal relevance. Further pharmacological and toxicological studies are recommended to develop a safe and effective antimicrobial formulation.

Keywords: *P. granatum*, Phytochemical Screening, Antibacterial, Flavonoids, Zone of Inhibition.

INTRODUCTION

Punica granatum L., commonly known as pomegranate, is cultivated across many regions, with the largest production occurring in Western Asia and the Mediterranean basin [1]. The fruit consists of peel, arils, and seeds in approximate ratios of 50%, 40%, and 10%, respectively [2]. Industrial processing generates nearly 60% by-products, much of which is discarded, leading to disposal challenges [3]. These residues, particularly the peel, are rich in secondary metabolites with significant potential as natural agents against microbial resistance [4]. Pomegranate and its products such as fresh and processed juice serve not only as nutrient-dense foods but also as valuable raw materials in pharmaceutical manufacturing [5]. The peel contains high levels of sugars, phenolic compounds, minerals, and organic acids notably, flavonoids and tannins, which are associated with multiple health benefits due to their influence on biochemical and enzymatic processes. Increasing awareness of natural functional ingredients has driven interest in incorporating dietary fiber, vitamins, probiotics, prebiotics, and other bioactive components into everyday diets [6, 7]. Earlier studies have reported the antimicrobial properties of pomegranate peel against pathogens including *Fusarium sambucinum*, *Escherichia coli*, *Bacillus subtilis*, and *Penicillium italicum* [8]. The seeds, arils, and peel are also abundant in polyphenols, flavones, anthocyanins, and catechins, contributing to strong antioxidant capacity [9]. Additionally, the high flavonoid and polyphenol content suggests potential anticancer applications [10]. Phytochemical evaluation in the present study showed that seeds contain polyphenols, ellagic acid, flavonoids, fatty acids, tannins, vitamin C, vitamin K, potassium, and folate; leaves contain quercetin, kaempferol, luteolin, tannins, anthocyanins, saponins, phenolic acids, vitamin C, and steroids; the peel has polyphenols, ellagic acid, alkaloids, gallic acid, punicic acid, saponins, tannins, vitamin C, and vitamin E; and stem bark contains flavonoids, alkaloids, gallic acid, punicic acid, saponins, tannins, vitamin C, and vitamin E [11-13]. Gram-positive *S. aureus* is among the most dangerous species of the Staphylococcus family [14], [15] very often cause skin infections and may cause pneumonia, heart valve infections, and bone infections which may be resistant to treatment with some antibiotics [16]. The increasing issue of antibiotic resistance and the growing interest in alternative remedies make the development of new antimicrobial medications derived from plants essential [1]. In addition to its nutritional and medicinal qualities, pomegranates, or *Punica granatum*, have long been used to cure infections, diarrhea, and inflammation. Its peel, seeds, leaves, and bark are home to phytochemicals with antibacterial and antioxidant properties, including tannins, flavonoids, and phenolics. Methanol is an effective solvent for these compounds. However, there is little comparative study of the phytochemical and antibacterial characteristics of the different parts of the pomegranate plant. The development of natural medications and the identification of the most bioactive components rely on these types of studies [17].

MATERIALS AND METHODS

Plant Material

The fresh *Punica granatum* (pomegranate) fruits were procured from the local market in Lucknow, India. The parts selected for the study included the peel, seeds, leaves, and stem bark. Each part was properly washed with running tap water to remove debris, followed by rinsing with distilled water. Samples were shade-dried at ambient temperature for 20–25 days until constant weight was achieved, and then ground into coarse powder using grinder. The powdered material was stored in airtight containers for extraction [18, 19].

Reagents and Chemicals

Methanol (95%), distilled water, Dragendorff's reagent, dilute sodium hydroxide and hydrochloric acid, ferric chloride solutions (1% and 5%), chloroform, concentrated sulfuric acid, glacial acetic acid, iodine solution, nutrient agar, among other reagents were used. The Staphylococcus aureus strain (MTCC No. 3160) was obtained in freeze-dried form from the MTCC (Microbial Type Culture Collection) at the IMTECH (Institute of Microbial Technology), Chandigarh, India, and was cultured following standard microbiological protocols.

Equipment

Weighing balance, B.O.D. incubator, laminar air flow chamber, Inoculation Loop, petri dishes, water bath, autoclave, sterile pipettes, reflux setup, steam distillation equipment, and mixer grinder.

Preparation of Plant Extract

Fresh plant parts of *Punica granatum* (peel, seeds, leaves, and stem bark) were shade-dried, then ground into a fine powder using a mechanical grinder. For each part, 100 g of the powder was placed in clean conical flasks and extracted with analytical-grade methanol at a ratio of 1:10 (w/v). The mixtures were kept at room temperature for 72 hours with occasional shaking to improve solvent penetration. After maceration, the extracts were filtered first through muslin cloth and then through Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using water bath at 40–50°C, to prevent heat-induced degradation. The crude methanolic extracts were stored in sterile airtight vials at 4°C until subjected to phytochemical screening and antimicrobial testing. This method maximized the yield and preserved the integrity of bioactive compounds [20, 21].

Phytochemical Screening

Methanolic extracts of *Punica granatum* parts were qualitatively analysed for the presence of flavonoids, saponins, tannins, phenols, terpenoids, alkaloids, and glycosides using standard test protocols and standardised procedures by Shihata (1951) [22].

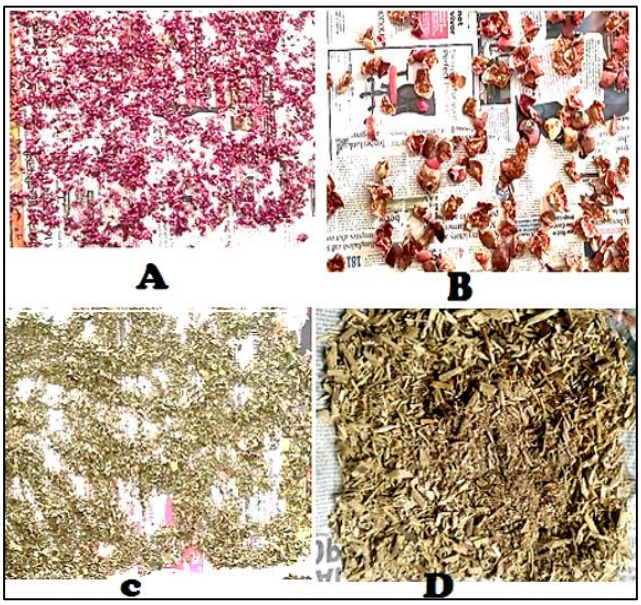

Figure 1: Pomegranate dried plant part: A. Seed, B. Peel, C. Leaf, D. Stem bark

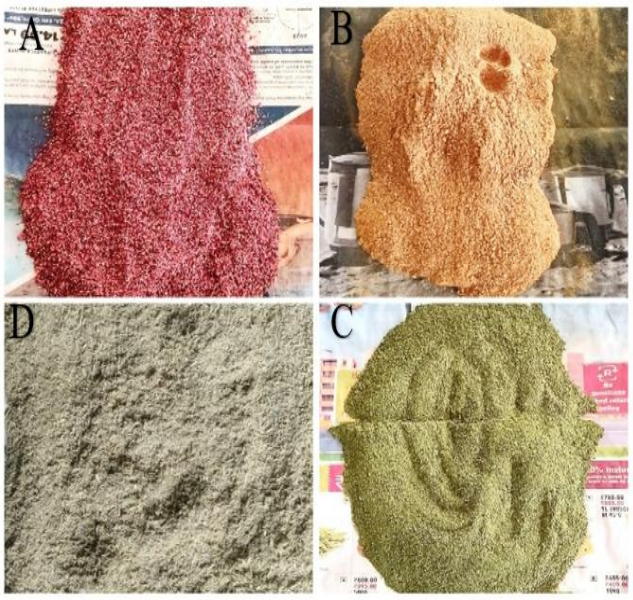

Figure 2: Powder form of pomegranate plant part: A. Seed, B. Peel, C. Leaf, D. Stem bark

Figure 3. Methanolic extract of different part of pomegranate plant

Preparation of Microbial Cultures

For antibacterial evaluation, the pure culture of the bacterial strain *Staphylococcus aureus* was selected due to its clinical relevance and known resistance potential. The strain was maintained in nutrient broth and incubated at 37 °C for 24 hours to ensure the development of actively growing inoculate. This sub-culturing step was essential for maintaining bacterial viability and ensuring standardized microbial load during the assay [23, 24].

Preparation of Agar Plates

MHA (Mueller–Hinton agar), the standard medium for antimicrobial susceptibility testing, was prepared according to manufacturer instructions and sterilized by autoclaving at 121 °C for 15 minutes. The molten medium was poured into sterile petri dishes under aseptic conditions and allowed to solidify at room temperature. Once solidified, the surface of each plate was inoculated with a fresh bacterial suspension prepared from the overnight culture. Sterile cotton swabs were used to spread the inoculum evenly across the entire agar surface, ensuring uniform bacterial lawn formation [25, 26].

Well Preparation

After inoculation, wells with a uniform diameter of 5–6 mm were carefully punched into the agar using a sterile cork borer. The agar plugs were removed aseptically to avoid contamination, and the wells were labeled [27, 28].

Application of Plant Extracts and Controls

Methanolic extracts of *Punica granatum* peel, seeds, leaves, and stem bark were prepared at a concentration of 100 mg/mL. Each extract was introduced into its designated well in a fixed volume of 100 µL using a micropipette. Penicillin G, a broad-spectrum antibiotic, served as the positive control to benchmark antimicrobial efficacy, while pure methanol was used as the negative control to rule out solvent interference. Care was taken to avoid spillage or overfilling, ensuring consistent test conditions across all plates [29].

Incubation

The prepared plates were incubated in an inverted position at 37 °C for 24 hours in a bacteriological incubator. Inversion minimized the risk of condensation droplets falling onto the agar surface, which could otherwise interfere with the diffusion of test samples [30].

Measurement of Zone of Inhibition (ZOI)

After incubation, the plates were observed for clear, circular inhibition zones surrounding each well. The diameter of each zone was measured in millimeters using a calibrated ruler or Vernier caliper. Each measurement was performed in triplicate to ensure reproducibility and accuracy. Larger inhibition zones were considered indicative of higher antibacterial efficacy [31, 32].

Data Analysis

The recorded zone of inhibition (ZOI) values for each plant extract were compared with that of the positive control. Results are presented as mean ± standard deviation, and percentage inhibition relative to ciprofloxacin was calculated. Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. All analyses were carried out using GraphPad Prism software version 8.02 [33].

RESULTS

In the present study, the antibacterial efficacy of methanolic extracts from the seed, peel, leaf, and stem bark of *Punica granatum* was evaluated against *S. aureus* using the agar well diffusion method, with measuring the zone of inhibition (ZOI) in centimetres to assess antimicrobial activity. The study provides insight into the comparative antibacterial properties of different plant parts, supporting their traditional medicinal use.

Phytochemical Screening

Methanolic extracts from different anatomical parts of *Punica granatum* - namely the leaf, seed, peel, and stem bark - were subjected to qualitative phytochemical screening. The analysis revealed the consistent presence of alkaloids, tannins, flavonoids, saponins, and phenols in all tested samples, suggesting their possible contribution to antimicrobial efficacy. The leaf extract exhibited the most diverse phytochemical profile, showing positive results for nine out of ten tested groups, including the presence of glycosides. Conversely, the peel extract lacks terpenoids, triterpenoids, steroids, and glycosides; however, its substantial levels of tannins, flavonoids, and phenols likely contributed to its strong antibacterial effect. Seed and stem bark extracts showed comparatively fewer positive phytoconstituents, which was correlated with their relatively lower antimicrobial activity. These findings reinforce the ethnomedicinal relevance of *P. granatum*, particularly the peel and leaf, and indicate that further pharmacological investigation of these plant parts could yield promising antibacterial agents.

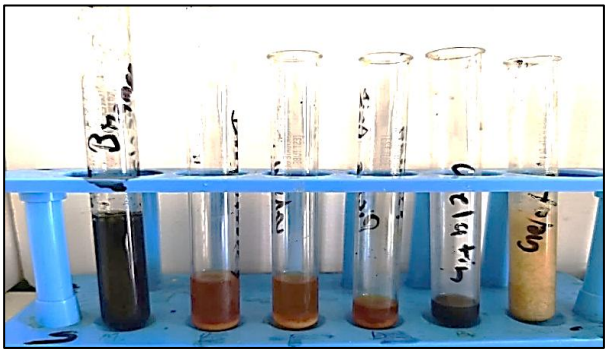

Figure 4: Test Results of Phytochemical Screening

Table 1: Phytochemical screening of methanolic extracts of *P. granatum*.

SN	TEST	LEAF	SEED	PEEL	STEM BARK
1	Alkaloids Test	+ve	+ve	+ve	+ve
2	Carbohydrate	+ve	+ve	-ve	-ve
3	Tannins Test	+ve	+ve	+ve	+ve
4	Flavonoids Test	+ve	+ve	+ve	+ve
5	Terpenoids Test	+ve	+ve	-ve	+ve
6	Triterpenoids Test	+ve	+ve	-ve	-ve
7	Saponins	+ve	+ve	+ve	+ve
8	Steroids	+ve	+ve	-ve	-ve
9	Phenols	+ve	+ve	+ve	+ve
10	Glycosides	+ve	-ve	-ve	-ve

Antimicrobial Screening

The antibacterial potential of the methanolic extracts was evaluated using the agar well diffusion method, and the inhibition zones were measured in centimetres. The standard drug (Penicillin G) displayed the highest inhibitory effect with a ZOI of 3.2 cm, serving as the positive control benchmark for comparative assessment.

Among the plant-derived extracts, the peel extract demonstrated the most pronounced inhibitory activity (2.2 cm), suggesting the presence of high levels of bioactive compounds with strong antibacterial properties. The leaf extract produced a moderate inhibition zone of 1.8 cm, closely followed by the seed extract at 1.7 cm, indicating a comparable but slightly reduced activity. The stem bark extract exhibited the lowest ZOI (1.5 cm), implying either reduced concentrations of active phytochemicals or the presence of compounds with less affinity for the bacterial target sites.

The differences in ZOI measurements were visually evident when comparing the clear inhibition halos surrounding the wells. The peel extract's larger halo was distinctly sharper and more defined, indicative of a potent and diffusive antibacterial effect. The relatively smaller halos for leaf, seed, and stem bark extracts point towards limited permeability or slower diffusion rates of the active compounds within the agar medium.

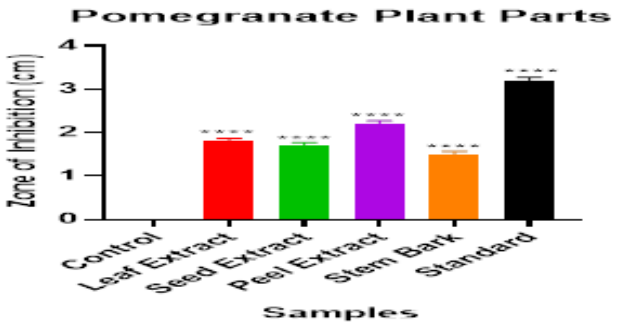

Figure 5: Different extracts of Pomegranate Plant Parts. All data were analysed *via* two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons tests using GraphPad Prism software. Data presented are Mean ± SD (n =5). ****P<0.0001 when compared to the control group.

Table 6: Antimicrobial Activity of various plant part extracts

S.N.	Groups Name	Zone of Inhibition (cm.)
1	Standard Group	3.2cm
2	Leaf Extract	1.8cm
3	Seed Extract	1.7cm
4	Peel Extract	2.2cm
5	Stem bark Extract	1.5cm

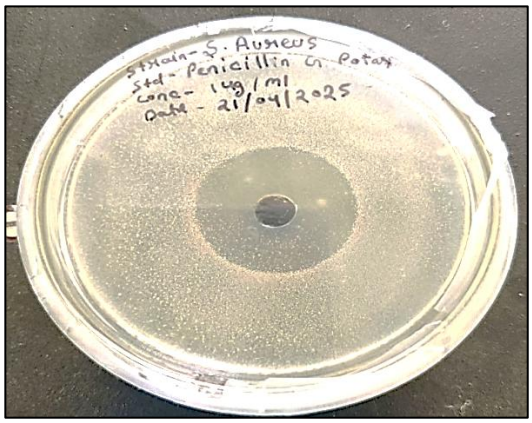

Figure 6: ZOI of standard antibiotic drug (Penicillin G potassium)

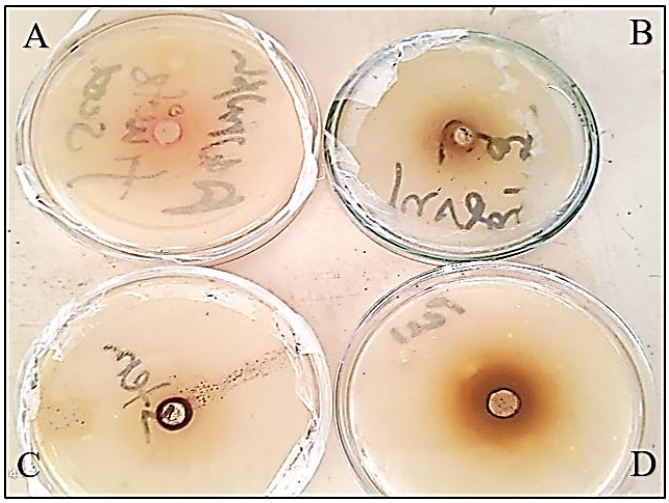

Figure 7: ZOI of different parts of pomegranate plant: A. Seed, B. Leaf, C. Stembark, D. Peels

DISCUSSION

The comparative analysis of antibacterial activity revealed a clear gradient in efficacy among the tested samples, with the standard antibiotic group exhibiting the largest zone of inhibition (3.2 cm). This result was anticipated, as the reference drug used penicillin G is a well-established broad-spectrum antibiotic with a high affinity for bacterial cell wall synthesis inhibition. Its superior activity provided a reliable benchmark for evaluating the performance of the *Punica granatum* extracts.

The peel extract exhibited the highest antibacterial activity (2.2 cm), likely due to its rich content of bioactive polyphenols, including punicalagin, ellagic acid, and flavonoids. These compounds can disrupt bacterial membranes, inhibit key enzymes, and induce oxidative stress, supporting its traditional use and potential as a natural antimicrobial source.

Leaf (1.8 cm) and seed (1.7 cm) extracts exhibited moderate antibacterial activity, likely due to flavonoids, phenolic acids, and tannins present in lower concentrations than the peel. These compounds act synergistically to inhibit bacterial growth, with similar inhibition zones indicating comparable potency despite potential differences in phytochemical composition. The stem bark extract showed the smallest inhibition zone (1.5 cm), reflecting the lowest antibacterial activity.

CONCLUSION

The most potent antibacterial activity is seen in the methanolic extract of pomegranate peel. This illustrates how natural antibacterial compounds can be made from peel waste. The peel exhibits the highest antimicrobial potency, followed by the leaf, seed, and stem bark in decreasing order. Because of its high tannin and phenolic content, the peel in this study stands out as the most promising plant part for antibacterial applications. The fact that all plant portions exhibited some level of activity supported *Punica granatum's* traditional medical use by demonstrating that it contains bioactive components with antibacterial potential. The findings urge for further study on the identification and quantification of specific active components and support the traditional uses of *Punica granatum*, especially the peel.

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